Sex Steroids and Heart Rate Variability in Patients after Myocardial Infarction

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Background: Although the relationship between sex steroid levels and coronary artery disease (CAD) has been the subject of many studies there are still controversies concerning the role of sex steroids in CAD. In patients with CAD, especially after a myocardial infarction, there is evidence for autonomic nervous system dysfunction. However, there is no data detailing the relationship between sex steroids and cicardian autonomic activity in patients with CAD. The aim of the study was to evaluate the association between sex steroids and heart rate variability (HRV) parameters in postinfarction patients.

Methods: In 88 postinfarction men (aged 36–73, average 53 years), 24-hour Holter monitoring was performed to assess HRV parameters: SDNN, SDNNI, SDANN, rMSSD, pNN50, and levels of the following hormones were measured: testosterone, estradiol, free testosterone index, and estradiol/testosterone ratio. Univariate and multivariate regression analyses were used to investigate the relationship between HRV parameters and levels of tested hormones.

Results: Increased testosterone levels were associated with increased SDNN ($r = 0.38$, $P = 0.03$), increased rMSSD ($r = 0.51$, $P = 0.002$), and increased pNN50 ($r = 0.45$, $P = 0.007$). These associations remained significance after adjustment for age, ejection fraction, and other relevant clinical covariates. There was no significant association between estradiol and HRV parameters.

Conclusion: In men with a history of myocardial infarction, higher levels of testosterone are associated with higher HRV measures of parasympathetic activity. These findings suggest that testosterone beneficially influences autonomic regulation of the heart. **A.N.E. 2004;9(2):156–161**

coronary artery disease; testosterone; heart rate variability; myocardial infarction

Although the relationship between sex steroid levels and coronary artery disease (CAD) has been the subject of many studies, there are still controversies concerning the role of sex steroids in CAD.[1–4] There are studies which have documented lower levels of testosterone in men with CAD[5,6] and other which did not find a significant association between serum testosterone and CAD.[2,3,7,8] The influence of testosterone on CAD risk factors remains controversial as well. Both positive[9] and negative[10] correlations between testosterone concentrations and HDL-cholesterol levels have been documented.

A positive correlation was found between testosterone levels in blood and profibrinolitic plasma activity in men.[11] Additionally, it was proposed that testosterone supplementation reduced CAD symptoms and improved exercise tolerance as well as improved coronary flow.[12–15] Other studies have described negative testosterone effects on coronary arteries. Coronary atherosclerosis was observed to be more extensive in testosterone-treated animals (monkeys) relative to the untreated controls.[16] It has also been shown that the testosterone treatment enhanced coronary artery vasoconstriction in guinea pigs.[17] Due to these controversies, testosterone supplementation in CAD patients is still not the standard of care.

Our recent study has revealed that estradiol and testosterone could promote atherogenesis.[18,19] We documented a positive correlation between the free

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testosterone index (the index permits the evaluation of bioactive testosterone which is not conjugated with blood proteins) and LDL-cholesterol levels in men with CAD. We observed the same correlation for estradiol, which supported results of other studies.[20] These observations may point to a negative role of testosterone and estradiol in the advance of atherosclerosis.

Though the importance of sex steroids in CAD pathogenesis is still discussed, the role of the autonomic nervous system in CAD is better documented. Dysfunction of the autonomic regulation of heart rate is well described in patients after myocardial infarction.[21,22] Heart rate variability (HRV) is a noninvasive method of assessing the autonomic tone.

There are studies reporting the testosterone influence on autonomic nervous function.[23–25] Testosterone intensifies emotional reactions and aggression, provoking a faster heart rate. Rejeski et al.[26] described an increase in aggression after intravenous testosterone infusion in monkeys. The correlation between testosterone levels in blood and emotional reactions has been proven in both men and women.[27–29] Estradiol influences the autonomic nervous system function as well.[23,30,31] So far there is no work detailing the relationship between sex steroids and cicardian autonomic activity in patients with CAD.

The aim of our study was to investigate the relationship between the total testosterone level, free testosterone index, estradiol level, estradiol/testosterone ratio, and HRV parameters in the group of men after myocardial infarction.

METHODS

Study Population

The study population consisted of 88 men aged 36–73 years (mean age: 56 years) with CAD (presence of coronary arteriosclerosis was documented by angiography) after myocardial infarction. Enrollment criteria included 88 consecutive CAD patients seen by a physician in the outpatient clinic. All the patients were in stable CAD (I–II grade according to the Canadian Cardiac Society class). Exclusion criteria were as follows: nonsinus rhythm (atrial fibrillation, paced rhythm), coexisting valvular heart disease, unstable angina, myocardial infarction less than 3 months, history of CABG, and heart failure symptoms III–IV NYHA class.

Hormonal Studies

Estradiol and testosterone blood levels were collected. There were two samples gathered in a 30-min interval (in the morning between 07.00 and 08.00) in order to avoid short time and circadian hormone concentration changes. After centrifugation, the plasma taken from the samples was mixed, and then hormone concentrations were measured. Every hormone concentration was determined with the use of the same reagents. Up to the assay, plasma was stored at the temperature of $-20\,^{\circ}C$, not longer than 60 days. Estradiol and testosterone levels were determined by an ORION radioimmunological assay. Free testosterone index and estradiol/testosterone ratio were also determined.

Heart Rate Variability Analysis

All the patients had 24-hour Holter ECG monitoring using 3-channel Oxford MR3 4500 tape recorders. The CS-2, CM-5, and IS leads were used. The scanning of recordings was done by Oxford Medilog Excel 2 system, then data were verified according to American Heart Association and American College of Cardiology recommendations.[32] In order to avoid HRV/drug influences, beta-blockers were withdrawn 48-hours before the recording onset.[12]

The following HRV parameters were computed: SDNN, SDNNI, SDANN, rMSSD, pNN50. The parameters were calculated according to the following definitions: SDNN—standard deviation of all recorded NN intervals; SDNNI—mean of the standard deviations of all NN intervals for all 5 min segments; SDANN—standard deviation of the average NN intervals in all 5 min segments; rMSSD—root square of the mean of the sum of the squares of the differences between adjacent NN intervals; pNN50—number of pairs of adjacent NN intervals differing by more than 50 ms divided by the total number of all NN intervals. Frequency domain HRV parameters were not tested, since there is a limited usefulness of such parameters in ambulatory (noncontrolled) conditions, and since program for such analysis was not reliable.

Statistical Analysis

Data are displayed as mean $\pm$ standard deviation (SD) values for continuous variables. The Shapiro-Wilk test was used to evaluate the distribution of data. Spearman test was used for the analysis of

correlations. The significance of group differences was determined by the Mann-Whitney test. Univariate and multivariate analyses were performed to determinate the differences between the groups. For all statistical calculations Statistica software for Windows (StatSoft, Inc. 1996, Poland) was used.

RESULTS

Clinical Characteristics

All patients have had prior myocardial infarction, with time from the infarction to the study enrollment ranging between 1 and 15 years (median: 8 years). Two-thirds of the patients suffered from a Q-wave infarction and five patients had two infarcts. About half of patients had anterior location of myocardial infarction. Left ventricle ejection fraction was evaluated by two-dimensional echocardiography and in the studied group ranged from 36% up to 70% (mean: 55% ± 10%). There were 13 (15%) active smokers and 21 patients (24%) who stopped smoking not longer than 5 years before. There were 37 (42%) hypertensive patients and 12 (14%) diabetics. Over 60% of patients were obese (BMI > 25 kg/m^2) with a mean BMI of 27 ± 3 kg/m^2.

Hormone Levels and HRV Parameters

Hormone levels and HRV values are presented in Table 1. Blood plasma testosterone concentration ranged from 6.8 to 59.4 nmol/l, median: 18.4 nmol/l. Statistically significant positive correlations were found between testosterone concentration and rMSSD ($r = 0.51$; $P = 0.002$), pNN50 ($r = 0.45$, $P = 0.007$), as well as with SDNN and SDNNI (Table 2 and Fig. 1). There was no significant association between HRV parameters and other hormones tested.

In the univariate analysis, variables significantly associated with higher level of testosterone (dichotomized at median > 18.4) were history of hypertension, SDNNI, rMSSD, pNN50 (Table 3 and Fig. 2). In the multivariate logistic regression analysis, SDNNI, rMSSD, and PNN50 remained statistically significantly associated with testosterone levels after adjustment for the clinical variables (Table 3).

DISCUSSION

Disturbances of autonomic balance are frequently observed in patients after myocardial infarction in which sympathetic activation and parasympathetic withdrawal have been observed. This autonomic imbalance contributes to an increased risk of congestive heart failure and mortality in postinfarction patients.[21,22,33–35] Our study was the first to document a significant association between testosterone levels and HRV parameters, reflecting autonomic control of the heart. Higher levels of HRV parameters reflecting parasympathetic tone were associated with higher levels of testosterone. Parasympathetic dominance markers differentiated the group of patients with testosterone concentrations above the median from the group of patients with testosterone levels below the median. These results are in agreement with previous experimental work, however, clinical data are lacking.[24,25,36] The central nervous system is a potential place for switching the hormone signal to

Table 1. Hormone Concentrations in Blood and Values of Heart Rate Variability Parameters

Variables	Mean	SD	Minimum	Maximum	Median
Age	55.2	8.8	36.0	73.0	53.4
Testosterone (nmol/l)	19.8	8.7	6.8	59.4	18.5
FTI	0.4	0.2	0.1	0.8	0.4
Estradiol (pmol/l)	94.0	59.5	22.1	429.6	80.7
E/T	5.4	3.5	1.0	18.2	4.2
SDNN	129.1	32.1	60.1	200.9	130.0
SDNNI	54.1	19.3	14.5	117.4	52.3
SDANN	114.5	31.0	51.6	189.0	115.3
RMSSD	32.2	20.6	9.6	133.5	28.1
pNN50	6.7	6.3	0.1	23.4	5.1

SD—standard deviation, FTI—free testosterone index, E/T—estradiol/testosterone ratio.

Table 2. Correlation Coefficient (r) Between Sex Hormone and Heart Rate Variability (HRV) Parameters (Spearman test)

HRV Parameters	Testosterone		Estradiol		E/T		FTI	
	r	P	r	P	r	P	r	P
SDNN	0.38	0.03	0.11	0.54	0.08	0.65	0.27	0.14
SDNNI	0.43	0.01	−0.06	0.71	−0.25	0.15	0.09	0.6
SDANN	0.31	0.07	0.13	0.48	0.06	0.73	0.3	0.09
rMSSD	0.51	0.002	0.13	0.47	−0.1	0.56	0.18	0.32
pNN50	0.45	0.007	0.05	0.78	−0.02	0.36	0.21	0.24

E/T—estradiol/testosterone ratio; FTI—free testosterone index, $P < 0.05$—considered statistically significant.

a neural signaling. Testosterone-dependent promotion of parasympathetic dominance may come from its positive effect on coronary blood flow. Vasodilating testosterone activity was described by Webb et al.[14] Testosterone-specific androgen receptors are described in the primates brain.[37] The work by El Mas et al.[24] gave indirect proof for testosterone-dependent changes in the animal autonomic nervous system. These authors described testosterone-dependent baroreflex modulation. It was also assumed that testosterone is a stronger supporter of barorelfex in males than in females.[25]

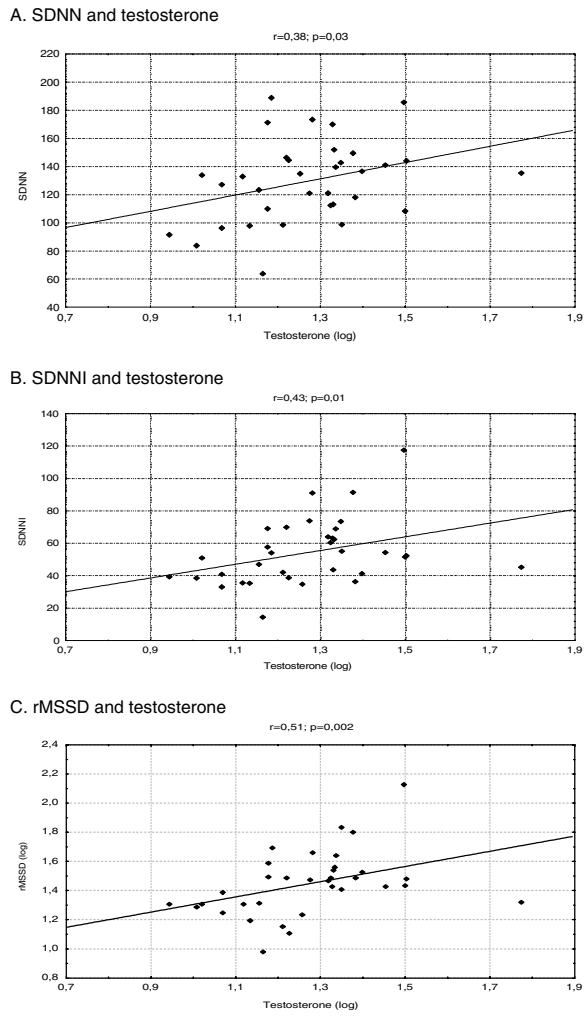

Figure 1. Relationship between testosterone and HRV parameters: SDNN, SDNNI, rMSSD (r from Spearman test).

There are some suggestions that heart rate changes may be partly controlled by cardiovascular androgen receptors for testosterone. Bricout et al.[38] described androgen receptors in the rabbit heart.[39] Although receptors for the suprarenal androgen-dehydroepiandosterone are known,[40] testosterone-specific receptors have not yet been found in the human heart. Hartman et al.[41] have detailed the direct testosterone influence on sympathetic heart fibers in mice.

Testosterone is transformed to estradiol in target tissues, and many of its effects (especially in the cardiovascular system) may depend on this transformation. Estradiol concentration in men after myocardial infarction is higher than in CAD men without prior myocardial infarction.[42,43] Moreover, reduced total testosterone concentration and higher estradiol/testosterone ratio were reported after myocardial infarction.[44] Estradiol influences cardiovascular regulation positively.[31] Animal studies suggested that estradiol could stimulate parasympathetic activity.[23,45] Baroreflex modulation by estradiol of central origin was detected in animals.[46]

Our study does not confirm the existence of a relationship between the blood estradiol level and autonomic activity. Tissular conversion from testosterone to estradiol in the central nervous system has a small influence on the estradiol level. Therefore, the level of estradiol circulating in the blood, opposite to testosterone, may not reveal its connection with autonomic activity in men.

Table 3. Univariate and Multivariate Analysis of Clinical Variables and Heart Rate Variability Parameters in Relationship to Testosterone Level Dichotomized at Median Value (>18.4 nmol/l)

	Univariate			Multivariate*	
Variables	**T ≤ 18.4 nmol/l**	**T > 18.4 nmol/l**	**P**	**OR**	**P**
Age (years)	56	55	0.64		
Anterior MI	36%	40%	0.68		
Hypertension	75%	25%	0.02	1.42	0.03
Diabetes	50%	51%	0.96		
Smoking	51%	50%	0.95		
HRV					
SDNN	122	139	NS		
SDNNI	45	63	<0.01	1.54	0.004
SDANN	111	119	Ns		
rMSSD	23	42	<0.01	1.47	0.01
pNN50	4.3	9.6	<0.01	1.53	0.006

Median values are shown for continuous parameters. NS—nonsignificant; T—testosterone; OR—odds ratio.
*HRV parameters added one at a time.

This study provides insight into the mechanistic link between testosterone levels and autonomic nervous control of the heart. There is no direct clinical implication of the findings, since there is no clinical evidence that the supplementation with testosterone or its derivatives in patients with CAD is safe and effective. Future studies are needed to evaluate the safety and efficacy of such supplementation (with DHEA or testosterone) in CAD patients with low levels of testosterone.

In conclusion, the results of our study suggest that testosterone has a positive impact on autonomic tone in men after myocardial infarction by promoting parasympathetic dominance.

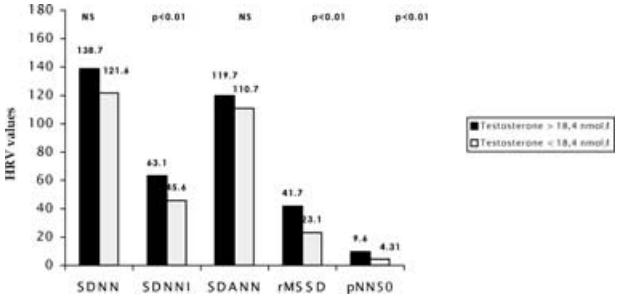

Figure 2. HRV parameters in relationship to low testosterone level (below median) and high testosterone level (above median). Median: 18.4 nmol/l (Mann-Whitney test).

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